

# High-Performance Computing Bioinformatics data analysis environment @ CINECA





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Cineca is the Italian e-Infrastructure for High Performance Computing and research data.

The mission of the HPC, SuperComputing Application and Innovation Department (SCAI) is to provide the national and EU community the state-of-the-art of High-Performance-Computing and Big-Data services.



Cineca provides HPC resources to Italian and European community research through peer reviewing of respectively ISCRA and Prace projects

Typical size per project

Italy

ISCRA C 50,000 core hours

ISCRA B 200,000 core hours



Europe





### Huge amount of sequencing data

With the advent of Next Generation Sequencing (NGS) platforms biological sequencing data are now produced in unprecedented amounts.

NGS Seq

#### NGS data size

HiSeq 2000 Output:

300 Gb (fastq)

375 Million/lane PE reads

Increased size due to replicates and PE

NGS applications are resource-hungry
This huge quantity of data requires computational
clusters Support of Computational Centers (e.g. CINECA)







#### Storage and processing of large volumes of data

Model: IBM NeXtScale

Architecture: Linux Infiniband cluster

Processor type: Intel Xeon E5 2670 v2 @2.5Ghz

Computing Nodes: 66+

Each node: 20 cores, 128 GB of RAM + 2 accelerators

Computing Cores: 1.320+

RAM: 6,4 GB/core

Disk space: 4 PB (+12 PB on tape)

Fast disk space: 40 TB (SSD)

#### plus

2 Visualization nodes (+2 Nvidia K40 GPUs each)

2 FAT nodes (512 GB of RAM + 1 GPU)

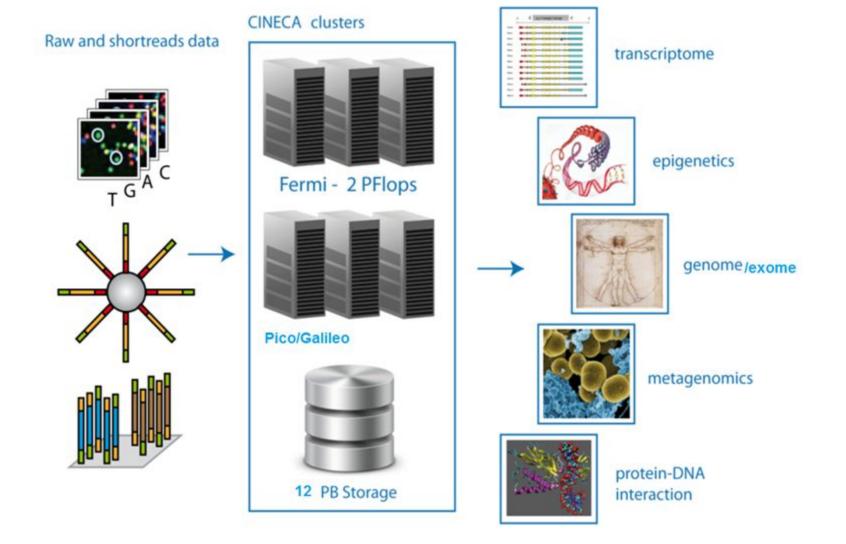
4 BigInsights nodes (32 TB of local disk)







### What does CINECA offer for bioinformatics?



#### 1. Computing resources

Bioinformatics software available through command line

#### 2. Advanced services

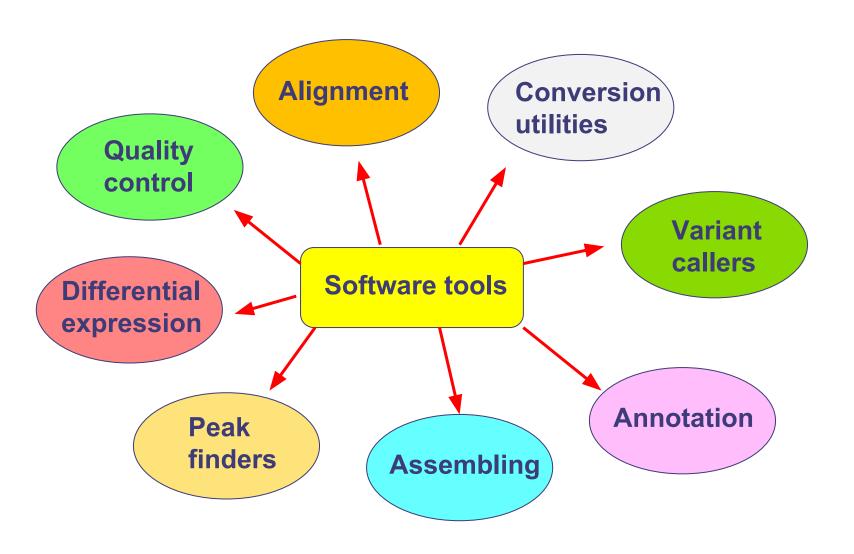
**Automated web workflows for Next Generation Sequencing** 

#### **3.** Bioinformatics Expertise

To customize solutions or implement new systems and tools



# Bioinformatics software available through command line





# Bioinformatics software available through command line

## **Quality** control

fastqc ngsqctoolkit trimmomatic

#### **Annotation**

annovar snpeff ngsrich

## Conversion utilities

samtools bedtools vcftools sra picard

#### **Alignment**

abra
diamond
bowtie
bwa
shrimp
tophat
blast+
mosaik
mauve
mummer
star
bismark

## General Purpose

bioconducto r biopython cluto igvtools idl mrjob R emboss



# Bioinformatics software available through command line

**RNA-Seq** 

cufflinks htseq splicetrap chimerascan reditools Peak finders

macs peakranger sicer Variant callers

gatk mutect varscan2 lofreq **Assembling** 

spades velvet ray cisa pagit

Metageno mics

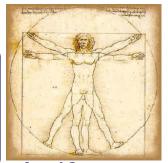
concoct giime

Cineca can add new software under user requests

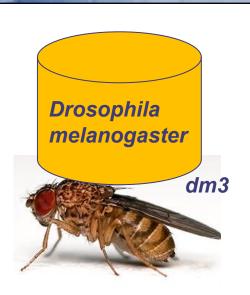


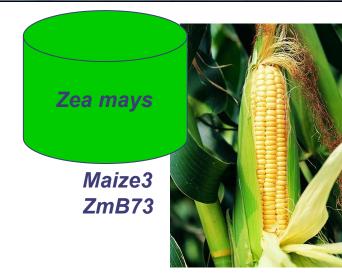
## Available released genomes

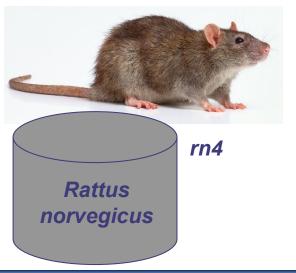


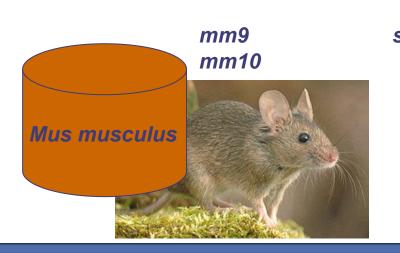


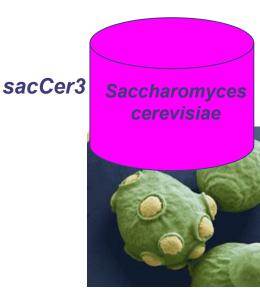
hg18 hg19 hg38









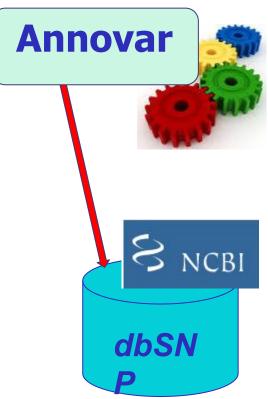


www.cineca.it

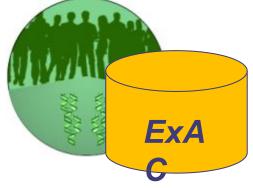


## Annotation databases

ANNOVAR is an efficient software tool to utilize update-to-date information to functionally annotate genetic variants detected from diverse genomes



Cineca can add new genomes and annotation databases under user requests



ExAC Data Set:
exome sequencing data
from a wide variety of
large-scale sequencing projects

A free public archive for short genetic variation within and across different species

• Please fill out the form on:

### https://userdb.hpc.cineca.it/user/register

- You'll receive userdb credentials: Then
- → Click on "HPC Access" and follow the on-screen instructions
- →You'll be asked to upload an image of a valid ID document
- → Ask your PI or send an email to <a href="mailto-superc@cineca.it">superc@cineca.it</a> to be included on an active project.

• When everything is done an automatic procedure sends you (via 2 separate emails) the username/password to access HPC systems

All cluster HPC infrastructures are available for bioinformatics.

PICO is the infrastructure dedicated to NGS bioinformatics applications and

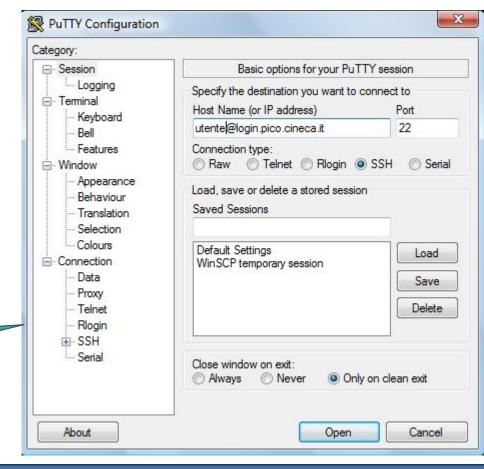
big data.

Users can access trough command line

scp, ssh for linux users
 (ssh username@login.pico.cineca.it)

 putty, winscp, TECTIA for windows users

Example of connection on the front-end *PICO* through putty application



#### SHOME

- Permanent, backed-up, and local.
- $\cdot$ Quota = 5GB.
- •For source code or important input files.

#### \$CINECA SCRATCH :

- Large, parallel filesystem (GPFS).
- •Temporary (files older than 30 days automatically deleted), no backup.
- •No quota max. A cleaning procedure for files older than 30 days

#### **\$WORK:**

Permanent, backed-up, project specific, 1 TB quota by default.



## Storage and file system

```
tcastign@node013.pico:[tcastign]$ cd $CINECA_SCRATCH tcastign@node013.pico:[tcastign]$ pwd /pico/scratch/userinternal/tcastign
```

```
tcastign@node013.pico:[tcastign]$ cd $HOME tcastign@node013.pico:[~]$ pwd /pico/home/userinternal/tcastign
```

```
tcastign@node013.pico:[tcastign]$ cd $CINECA_SCRATCH tcastign@node013.pico:[cin_staff]$ pwd /gpfs/work/cin_staff
```



## Accounting: saldo

Accounting philosophy is based on the resources requested for the time of the batch job:

cost = no. of cores requested x job duration

In the CINECA system it is possible to have more than 1 budget ("account") from which you can use time. The accounts available to your UNIX username can be found from the saldo command.

account totConsumed	start	end	total	localCluster	totConsumed	
e			(local h)	Consumed(local h)	(local h)	
 - try11_test	20110301	20111201	10000	0	2	

- CINECA's work environment is organized in modules, a set of installed libs, tools and applications available for all users.
- "loading" a module means that a series of (useful) shell environment variables wil be set

Bioinformatics applications, public databases and annotations are pre-installed on *PICO* cluster.

On *PICO* cluster *bioinformatics modules* can be easily called by using the "module" environment.

"module" environment allows the user, by using a single command, to:

- list all the installed programs
- list all the genomes, indexes, and annotation databases
- get all the configured path (set environmental variables)
- automatic load the program in any directory
- launch the program



## Module environment: usage

#### Command to initialize the module environment

\$ module load profile/advanced

#### Command to list the installed modules

\$ module available

#### Command to load a module program

\$ module load name\_program



### Module commands

- > module available (or just "> module av")
- Shows the full list of the modules available in the profile you're into, divided by: environment, libraries, compilers, tools, applications
- > module (un)load <module\_name>
  (Un)loads a specific module
- > module show <module\_name>Shows the environment variables set by a specific module
- > module help <module\_name>
  Gets all informations about how to use a specific module
- > module purge
  Gets rid of all the loaded modules



## Module environment: usage

#### Command example to list available modules in «profile bio»

```
$ module available
      ----- /cineca/prod/modulefiles/base/biodata ------
D melanogaster/dm3
                     Mus musculus/mm9
                                            Z mays/ZmB73
Homo Sapiens/hg18
                      R norvegicus/rn4
                                            Z mays/maize3
Homo Sapiens/hg19
                      S cerevisiae/sacCer3
                                            Mus musculus/mm10
Z mays/Mo17 v1(default)
       ---- /cineca/prod/modulefiles/base/applications ----
annovar/2014Sep15
                cufflinks/2.2.1
                                          snpeff/4.1b
bedtools/2.21.0
                  fastqc/0.11.2
                                          star/2.4.0d
bowtie/1.0.1
                    id1/8.1
                                          tophat/2.0.11(default)
bowtie2/2.2.3 picard/1.119
                                          tophat/2.0.12
bwa/0.7.10
                     samtools/0.1.19
                                          vcftools/0.1.12b
chimerascan/0.4.5a samtools/1.1
```



## Module commands

## > module available (or just "> module av") Examples

-----/cineca/prod/modulefiles/advanced/applications ------

bioconductor/2.14	1gmql/2.2	peakranger/1.17	tabix/0.2.6
bioconductor/3.0	homer/4.7	picard/1.119	tophat/2.0.11
biopython/1.65	htseq/0.6.1	pintron/1.3.0	tophat/2.0.12
bismark/0.14.2	idl/8.1	qiime/1.9.0	treetagger/3.2
blast+/2.2.30	igvtools/2.3.4	0 r/3.1.2	trimmomatic/0.33
bowtie/1.0.1	lofreq/2.1.1	r/3.2.2	ucsc/1.0
bowtie2/2.2.3	macs/1.4.0	racket/6.2.1	varscan2/2.3.7

> module available bowtie\*

-----/cineca/prod/modulefiles/advanced/applications ------

bowtie/1.0.1 bowtie2/2.2.3

- > module load bowtie2/2.2.3
- > module list

**Currently Loaded Modulefiles:** 

- 1) profile/advanced 2) bowtie2/2.2.3
- > module show bowtie2/2.2.3

\_\_\_\_\_\_

/cineca/prod/modulefiles/advanced/applications/bowtie2/2.2.3:

module-whatis Fast and sensitive read alignment setenv BOWTIE2\_HOME /cineca/prod/applications/bowtie2/2.2.3/binary prepend-path PATH /cineca/prod/applications/bowtie2/2.2.3/binary/bin

\_\_\_\_\_

## Module commands

#### > module help bowtie2/2.2.3

\_\_\_\_\_

Module Specific Help for /cineca/prod/modulefiles/advanced/applications/bowtie2/2.2.3:

modulefile "bowtie2/2.2.3"

bowtie2-2.2.3

Fast and sensitive read alignment

\_\_\_\_\_

License type: gpl

Web site: http://bowtie-bio.sourceforge.net/bowtie2/index.shtml

Download url: http://sourceforge.net/projects/bowtie-bio/files/bowtie2/2.2.3/

\_\_\_\_\_

Bowtie 2 is an ultrafast and memory-efficient tool for aligning sequencing reads to long reference sequences. It is particularly good at aligning reads of about 50 up to 100s or 1,000s of characters, and particularly good at aligning to relatively long (e.g. mammalian) genomes. Bowtie 2 indexes the genome with an FM Index to keep its memory footprint small: for the human genome, its memory footprint is typically around 3.2 GB. Bowtie 2 supports gapped, local, and paired-end alignment modes.

\_\_\_\_\_



## Module commands: dependencies

#### > module load biopython/1.65

WARNING: biopython/1.65 cannot be loaded due to missing prereq.

HINT: the following modules must be loaded first: python/2.7.8

• What happens?

#### > module show biopython /1.65

\_\_\_\_\_

/cineca/prod/modulefiles/advanced/applications/biopython/1.65:

module-whatis Biopython is a set of freely available tools for biological computation written in Python by an international team of developers.

#### prereq python/2.7.8

setenv BIOPYTHON\_HOME /cineca/prod/applications/biopython/1.65/gnu--

4.8.3

prepend-path PYTHONPATH /cineca/prod/applications/biopython/1.65/gnu--

4.8.3/lib/python2.7/site-packages



## Module commands: dependencies

- > module load autoload biopython/1.65
- > module list
  - **Currently Loaded Modulefiles:**
  - 1) profile/advanced 3) gnu/4.8.3
- 5) biopython/1.65
- 2) autoload/0.1 **4) python/2.7.8**
- > module show python/2.7.8

/cineca/prod/modulefiles/advanced/tools/python/2.7.8:

module-whatis python language

```
gnu/4.8.3
prereq
conflict python
```

PYTHON HOME /cineca/prod/tools/python/2.7.8/gnu--4.8.3 setenv

prepend-path PYTHONPATH /cineca/prod/tools/python/2.7.8/gnu--4.8.3/lib/python2.7/sitepackages:

prepend-path PATH /cineca/prod/tools/python/2.7.8/gnu--4.8.3/bin:

prepend-path LD LIBRARY PATH /cineca/prod/tools/python/2.7.8/gnu--4.8.3/lib:



### Module environment: load biodata

#### Command example to load available data and indexes

```
$ module load Homo Sapiens/hg19
several environment variables are defined:
$ module show Homo Sapiens/hg19
/cineca/prod/modulefiles/base/biodata/Homo Sapiens/hg19:
module-whatis
               Human Sapiens genome hg19
setenv GENOME /cineca/prod/biodata/Homo Sapiens/hg19/
              /cineca/prod/biodata/Homo Sapiens/hg19/annotation
setenv ANNOT
setenv GFASTA /cineca/prod/biodata/Homo Sapiens/hg19/genome
               /cineca/prod/biodata/Homo Sapiens/hg19/indexes
setenv GINDEX
setenv BWINDEX /cineca/prod/biodata/Homo Sapiens/hg19/indexes/bowtie-1.0.1
setenv BW2INDEX /cineca/prod/biodata/Homo Sapiens/hg19/indexes/bowtie2-2.2.3
that point to raw or indexed genomic data
```

## Module environment: usage

#### Command example to launch a program using environmental variables

```
S module load autoload bowtie2

$ bowtie2 $BW2INDEX/name_index -un output.unmapped.fastq --chunkmbs 128 -p 8 -k 1 --best -S input.sam --phred64-quals
```

## Lauching a job

• Now that we have our executable, it's time to learn how to prepare a job for its execution

Pico has the PBS scheduler.

• The job script scheme is:

- #!/bin/bash
- #PBS keywords
- variables environment
- execution line

### Environment setup and execution line

The execution line starts with ./myexe arg\_1 arg\_2:

arg 1 arg 2 are the normal arguments of myexe

The environment setting usually starts with "cd \$PBS\_O\_WORKDIR".

That's because by default you are launching on your home space the executable may not be found.

\$PBS\_O\_WORKDIR points to the directory from where you're submitting the job .

```
#PBS -N jobname
                                                           # name of the job
                                                           # output file
#PBS -o job.out
#PBS -e job.err
                                                           # error file
#PBS -l select=1:ncpus=20:mpiprocs=20:mem=122GB
                                                    # resources
#PBS -I walltime=1:00:00
                                                      # hh:mm:ss
#PBS -q <queue>
                                                      # chosen queue
                                                           # name of the account
#PBS -A <my account>
#PBS -W group list=<group>
                                                           # name of effective group
                                  for reservation
```

select = number of node requested
ncpus = number of cpus per node requested
mpiprocs = number of mpi tasks per node
mem = RAM memory per node



#### username@node013.pico:[~]\$

qsub -I -I select=1:ncpus=2:mpiprocs=1:mem=8GB -I walltime=5:00:00 -A train\_RNAseq15 -W group\_list=train\_RNAseq15 -q R121546

qsub: waiting for job 123456.node001 to start

qsub: job 123456.node001 ready

select = number of nodes requested
ncpus = number of cpus per node requested
mpiprocs = number of MPI tasks per node
mem = RAM memory per node
walltime = wall time limit
parallel = name of queue for parallel job (multithread too)
train... = account namec

#### username@node013.pico:[~]\$

qsub -I -I select=1:ncpus=2:mpiprocs=1:mem=8GB -I walltime=5:00:00 -A train\_RNAseq15 -W group\_list=train\_RNAseq15 -q R121546

qsub: waiting for job 123456.node001 to start

qsub: job 123456.node001 ready

username@node009.pico:[~]\$ module load profile/advanced

username@node009.pico:[~]\$ module load fastqc/0.11.3

username@node009.pico:[~]\$ fastqc --nogroup -t 2 --extract input.R1 input.R2 -o output



## Example of batch-script to launch fastqc/0.11.3 on PICO

```
#!/bin/bash

#PBS -N fastqc

#PBS -I select=1:ncpus=2:mpiprocs=1:mem=8GB

#PBS -q R121546

#PBS -I walltime=5:00:00

#PBS -A train_RNAseq15

#PBS -W group_list=train_RNAseq15
```

cd \$PBS O WORKDIR

==> change to current dir

module load profile/advanced module load fastqc/0.11.3

fastqc --nogroup -t 2 --extract input.R1 input.R2 -o output 2>&1 | tee input. log

## Example of batch-script to launch fastqc/0.11.3 on PICO

username@node013.pico:[~] qsub launch\_fastqc.sh

123456.node001



# Example of batch-script to launch fastqc/0.11.3 on PICO

```
#!/bin/bash
#PBS -N fastqc
#PBS -I select=1:ncpus=2:mpiprocs=1:mem=8GB
#PBS -q R121546
#PBS -I walltime=5:00:00
#PBS -A train RNAseg15
#PBS -W group list=train RNAseg15
INPUT HOME="/pico/home/userinternal/tcastign/test/input"
OUTPUT HOME="/pico/home/userinternal/tcastign/test/output"
OUTPUT FASTQC="/pico/home/userinternal/tcastign/test/output/fastgc"
echo $INPUT HOME;
echo $OUTPUT_HOME;
echo $OUTPUT FASTQC;
```

fastqc --nogroup -t 2 --extract \$INPUT\_HOME/\$fastq -o \$OUTPUT\_FASTQC 2>&1 |tee input.log

# Developing my first automated pipeline on PICO

### 1. Computing resources

### Bioinformatics software available through command line

PROS	CONS
Rich environment: bioinformatics resources continuosly updated	Basic Unix/Linux Knowledge needed
Flexible environment: Resources can be added under request depending on user needs	
Simple usage through «module» environment	

### **1.** Computing resources

Bioinformatics software available through command line

#### 2. Advanced services

**Automated web workflows for Next Generation Sequencing** 

**3.** Bioinformatics Expertise

To customize solutions or implement new systems and tools



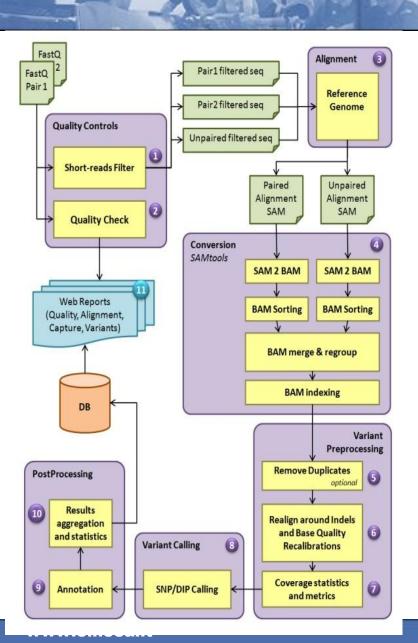
# **Bioinformatics NGS Pipelines**

Automated workflows (pipelines) for Next Generation Sequencing are available through a web interface and are able to perform analyses for several NGS application fields:

- Deep targeted exome sequencing;
- RNA sequencing (trascriptome analysis);
- Whole exome sequencing;
- Identification of DNA protein interactions by ChIP-seq;



### Ultra Deep Exome Sequencing Pipeline



# Online Deep Exome Sequencing Software Analysis (ODESSA)

Handles genes targeted at high coverage

Specifically focused for clinical diagnostics

Identifies (SNPs) and (DIPs) classified by different scores (e.g. depth, SIFT, MAV, MEQ).

Results are supported with genomic information, functional annotations, cross-linking databases and quality and relevance scores, graphics, tables and browsing, filtering and download.

Optimized for MiSeq Illumina platform





### Ultra Deep Exome Sequencing Pipeline

### **Example of output: variant results**

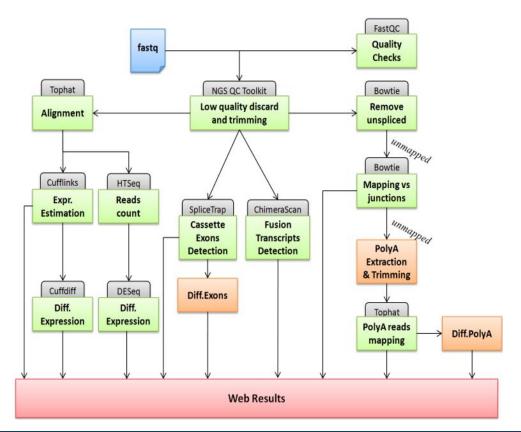
position	allele variation	state	Depth	Mutation	Туре	Func	gene info	location	dbSNP
chr16:23360199- 23360199	T→C	het	66	SNV	synonymous SNV		SCNN1B	exonic	rs238547
chr16:27373915- 27373915	$G\toT$	het	147	SNV	synonymous SNV	-	IL4R	exonic	rs2234898
chr16:85706047- 85706047	$A\toC$	het	62	SNV	synonymous SNV	-	GSE1	exonic	rs9940601
chr16:15818141- 15818141	$A\toC$	het	115	SNV	synonymous SNV	-	MYH11	exonic	rs2075511
chr16:89836323- 89836323	$C\toT$	het	140	SNV	nonsynonymous SNV		FANCA	exonic	rs7195066
chr16:20554248- 20554248	$G\toA$	het	166	SNV	synonymous SNV	-	ACSM2B	exonic	rs140717461
chr16:20489919- 20489919	$G\toA$	het	47	SNV	nonsynonymous SNV	-	ACSM2A	exonic	rs147314845
chr16:15811023- 15811023	$C\toT$	het	120	SNV	synonymous SNV	-	MYH11	exonic	rs1050163



## RNA Sequencing Pipeline

### The RNA-Seq Analysis Pipeline (RAP)

Performs a complete and customizable RNA-seq pipeline, allowing users to examine NGS data under many points of view:



- Gene and transcript expression
- Differential expression
- Splicing junctions
- Cassette exons
- Poly(A) sites
- Fusion transcripts

RNA editing



## **RNA Sequencing Pipeline**

#### Gene and transcript expression summary

Click on the colored-box numbers to open the expression overview

20	**********		Expressed	Expres	sed_	Expressed	Expressed	#HIDATA						
File	Label		FPKM>0	FPKM>		FPKM>20	FPKM>100	Loci						
1	Embryonic1	transcripts	22852	7374		4265	640							
		genes	16963	7180		4355	680	0						
2	Embryonic2	transcripts	23096	7436				Click o	on a column title to or	der this tol	ske			
		genes	17160	7196	UID	Gene	Transcript	Genomic Po	osition	Strand	TLen	#Exons	FPKM]	Coverage
3	Embryonic3	transcripts	23104	7332	1268	MIR4461	NR_039666	chr5:13429	1628-134291701	+	74	1	237307.93	9918.79
		genes	17160	7126	637	MIR548AC	NR_039621	chr17:28547	7066-28547096	-	31	1	64029.67	2676.26
4	Embryonic4	transcripts	23182	7408	987	MIR3687	NR_037458	chr21:16788	868-1678928	-	61	1	42134.91	1761,12
		genes	17223	7203	1206	MIR1267	NR_031671	chr4.177196	8342-177331125	*	57	3	39547.53	1652.97
5	Adult1	transcripts	23989	7198	672	MIR54802	NR_039605	chr17:6082	1546-60847231	-	52	3	34715.01	1450.99
		genes	17866	6987	941	MIR663A	NR_030386	chr20:26136	5822-26136914	*	93	1	16631.98	695.17
6	Adult2	transcripts	23874	7262	1282	MIR54802	NR_030385	chr5:159002	2885-159095000	+	81	4	14808.62	618.96
		genes	17782	7045	1214	MIR4454	NR_039659	chr5:73224	16-7322467	-	52	1	12569.28	525.36
					1603	MIR548D1	NR_030382	chr9.123415	5763-123798763	-	59	4	11998.16	501.49
w	ww.cineca	a.it			1207	MIR548AB	NR_039611	chr4:183713	3766-183720064	*	56	2	11737.12	490.58



### Whole-exome Sequencing Pipeline

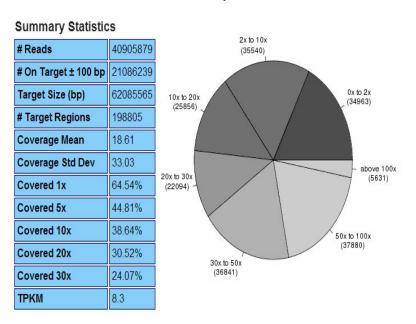
### Whole-Exome sequencing Pipeline (WEP)

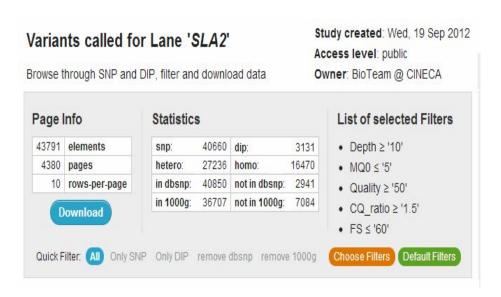
SNP and DIP detection and annotation

gapped alignment, duplicates removal, quality scores recalibration

cross-linking, intersections, trio analyses, statistics

#### Enrichment Performance of Sample file1.recal.bam.cleaned







## ChiP-seq Pipeline

### Chip-seq analysis pipeline (CAST)

- peaks detection
- peaks filtering
- peaks visualization on UCSC Genome Browser
- peaks annotation with genomic features

ummar	y Statistics Pe	aks Gene tools Formats for fu	ınctional analysis					
Lane	Label	Files	TOTAL Peaks	Corepromoter	Promoter	Genic	Downstream	Intergenic
<u>1</u>	HistoneK562 rep1	HistoneK562H3k4me3bUcd1.bam	781 <b>view</b>	296 view	432 view	131 view	153 view	(
2	HistoneK562 rep 2	HistoneK562H3k9acbUcd2.bam	2.789 view	5 view	692 view	1580 view	298 view	542 view
9	TfbsK562 input	TfbsK562InputStd1.bam	Control lane	-	-	-	-	
10	TfbsK562 rep 1	TfbsK562NfyaStd1.bam	1.508 view	4 view	168 view	729 view	78 view	616 view
11	TfbsK562 rep 2	TfbsK562NfybStd2.bam	1.505 view	10 view	186 view	682 view	113 view	625 view
12	TfbsK562 rep 3	TfbsK562Pol2Std2.bam	4.317 view	112 view	510 view	3023 view	767 view	624 view

#### 2. Advanced services

### **Automated web workflows for Next Generation Sequencing**

PROS	CONS
User-friendly graphic interface: The pipeline is completely automatized at each stage and doesn't require any computational knowledge by the user	Low flexibility: changes are allowed only with a specific project agreement with Cineca
Any knowledge of the underlying high-performance computing infrastructure is not needed by the user	
Automation avoids human errors introduced by hand-made scripts and also eases the processing of Big Data NGS experiments	

### **1.** Computing resources

Bioinformatics software available through command line

#### 2. Advanced services

**Automated web workflows for Next Generation Sequencing** 

### **3.** Bioinformatics Expertise

To customize solutions or implement new systems and tools



### Bioinformatics specialistic support

Cineca offers bioinformatics specialistic support to develop and optimize

- configuration parameters
- command-line programs
- complex bash scripts

on hundreds of computing cores

For further information write to: hpc-bioinformatics@cineca.it







#### **General Information**

Official web site http://www.hpc.cineca.it

Bio & Genomics http://www.hpc.cineca.it/content/hpc-bioinformatics

#### How to get computational resources?

ISCRA initiative http://www.hpc.cineca.it/services/iscra

PRACE: http://www.prace-ri.eu/

#### **Automated analysis workflows**

Target Exome https://bioinformatics.cineca.it/odessa

RNA-Seq https://bioinformatics.cineca.it/rap

Whole Exome https://bioinformatics.cineca.it/wep

ChIP-Seq https://bioinformatics.cineca.it/cast